American Journal of Epidemiology
Vol. 165, No. 9
DOI: 10.1093/aje/kwk085
Advance Access publication February 10, 2007

Human Genome Epidemiology (HuGE) Review

Genetic Effects versus Bias for Candidate Polymorphisms in Myocardial Infarction: Case Study and Overview of Large-Scale Evidence

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Received for publication May 16, 2006; accepted for publication November 5, 2006.

Several genetic polymorphisms have been proposed to be associated with myocardial infarction (MI). The authors examined the evidence and biases underlying such associations using a case-study meta-analysis and an overview of large-scale data. In a meta-analysis of 27 studies addressing the association of the angiotensin type 1 receptor (*AT1R*)+*1166A/C* polymorphism with MI (10,180 cases, 17,129 controls), the **C* allele conferred an increase in MI risk (odds ratio = 1.13 per allele, $p = 0.005$). However, there was large between-study heterogeneity; the largest study showed no effect, contradicting smaller studies; and studies with blinded genotyping showed no effect. The authors conducted an overview of meta-analyses of genetic associations for MI or coronary artery disease, including at least three studies and 3,000 subjects. In their latest meta-analysis, another 14 polymorphisms were found to have formally significant associations. If true, these associations would already explain 42% of the MI risk for Caucasian populations. Significant between-study heterogeneity was common. Across the 32 largest studies, only two found formally significant results (nine would be expected if each meta-analysis showed a true association). Even with large-scale evidence from meta-analyses, significant associations for MI may be subject to bias. Large-scale single studies and prospective consortia should be used for detecting and validating the genetic determinants of MI.

angiotensin II; *AT1R*; bias (epidemiology); epidemiology; genes; meta-analysis; myocardial infarction; polymorphism, genetic

Abbreviations: *AT1R*, angiotensin type 1 receptor gene; CAD, coronary artery disease; CI, confidence interval; MI, myocardial infarction; OR, odds ratio.

Editor's note: *This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

Myocardial infarction (MI) and coronary artery disease (CAD) may exhibit considerable heritability. This possibility is suggested by the importance of family history as a risk factor, the heritability of several other classic risk factors for these outcomes, and evidence from twin studies showing that 8–49 percent of the variance for a range of features of CAD may be inherited (1). This genetic component is likely to be determined by a number of different polymorphisms, each having a small effect but cumulatively influencing a sizable proportion of the MI and CAD risk (2). Until now, the search for these determinants has followed mostly the

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candidate gene approach. Genes involved in pathways of cardiovascular phenotypes have been evaluated in case-control studies for their association with MI and CAD outcomes. Many of these genes have given occasional signals for such associations in single studies, but these associations are often refuted in subsequent studies. Meta-analyses combining information from many studies may provide more conclusive answers regarding the presence of such associations (3–6). However, even with well-conducted meta-analyses, there is the threat that biases—for example, publication bias and selective reporting bias—may lead to spurious results. Several investigators have noted in some instances that large studies may give more conservative results than smaller studies (6–8), which may reflect bias in this literature.

Recently, the search for genetic determinants of MI and other common complex diseases and phenotypes has proceeded toward adopting discovery-oriented approaches with massive testing of polymorphisms (9, 10). At the same time, more and more promising associations with MI continue to appear at a rapid pace in prestigious journals (11, 12). It would be interesting to know how much of the total genetic variability we have explained already based on seemingly replicated associations to date. It is also useful to examine the extent to which biases may affect even the largest-scale evidence obtained from meta-analyses in this field.

Here, we set out to address these issues with empirical data. First, we performed a meta-analysis of 27 studies addressing one of the most commonly probed polymorphisms for its relation with MI, namely, the *+1166A/C* variant in the angiotensin type 1 receptor (*AT1R*) gene. Several small studies have suggested this polymorphism as a risk factor, but the largest study (8), with over 10,000 subjects, had entirely null findings. We performed the meta-analysis with a prime focus on detecting signals for potential biases. We also performed an overview of large-scale meta-analyses of genetic polymorphisms in which the results claimed a significant association with MI outcomes. We scrutinized these data for signals that would potentially reveal the presence of bias.

MATERIALS AND METHODS

Case-study meta-analysis

Study identification and eligibility. We considered all studies on the association of the *AT1R+1166A/C* polymorphism with MI. We searched PubMed (National Library of Medicine, Bethesda, Maryland) by using the following terms: angiotensin AND receptor AND (genetics OR genet* OR polymorphism OR mutation OR mutant OR allele OR variant OR SNP [single nucleotide polymorphism]) AND (myocardial infarction OR myocardial ischemia OR coronary OR heart OR cardiovascular) (last search, December 2005). We immediately excluded news, reviews, and meeting abstracts. We communicated with the authors of eligible reports when pertinent data were missing or when cardiovascular disease was assessed as the primary outcome and an MI subsample was included without explicitly presented data. In addition, family-based studies were excluded. To sharpen the definition of the phenotype under study, we also excluded studies focusing on CAD without separate data on MI outcomes.

Data extraction. From each study, we noted year of publication, country of origin, study design, proposed genetic contrast, information on genotypes and/or alleles, eligible and genotyped cases and controls, ''racial'' descent, gender, age, and MI definition. For studies including subjects of different ''racial'' descent, as well as for multicenter studies, data were extracted separately whenever possible. Furthermore, we examined whether any blinding or validation of genotyping had been used.

Among overlapping studies, only the largest was retained. Two investigators independently extracted the data; disagreements were discussed with a third arbitrator, and consensus was reached on all items.

Data synthesis. **C* allele frequencies were synthesized across all control groups with random-effects models. Conformity of controls' data with Hardy-Weinberg equilibrium was tested with an exact test. The primary analysis was based on the contrast of alleles. Secondary analyses examined dominant and recessive inheritance models. The odds ratio was used as the metric of choice. For each genetic contrast, we estimated the between-study heterogeneity across all eligible comparisons by using the χ^2-based Q statistic (significant for $p < 0.10$) (13). We also quantified the extent of heterogeneity with I^2 (range, 0–100 percent; values >75 percent imply extreme heterogeneity) (14). Data were combined by using both fixed- and random-effects (13) models. Fixed-effects models assume that there is a common genetic effect across all studies and that differences are due to chance. They have the advantage of being relatively insensitive to small studies with exaggerated spurious effects, but they are counterintuitive in the presence of heterogeneity. Random-effects models incorporate an estimate of the between-study variance and provide wider confidence intervals when results differ among studies. Given that heterogeneity is quite common in genetic association studies, as revealed by systematic appraisals of accumulated genetic information (3, 4, 7), random-effects models seem more appropriate. However, they may be influenced more by small studies with exaggerated spurious effects.

Subgroup, sensitivity, and bias analyses. We performed additional analyses on the allele-based data that aimed to dissect potential sources of heterogeneity and bias. We examined whether results changed when limited to studies with rigorous selection of cases and controls (e.g., World Health Organization criteria) or when limited to studies that reported any blinding. We performed cumulative and recursive cumulative meta-analysis (4, 15) to evaluate whether the summary odds ratio and between-study heterogeneity estimates changed over time. We used the Begg-Mazumdar test to evaluate whether the magnitude of the observed association was related to the variance of each study; that is, small studies gave different results than larger studies (16). We also compared the results of the largest study against the results of smaller ones (17). Finally, we examined the impact of correcting for deviations from Hardy-Weinberg equilibrium (18).

Overview of meta-analyses

Search for meta-analyses and eligibility criteria. We identified meta-analyses providing a quantitative synthesis for the relative risk conferred by the probed genetic polymorphism and that found a statistically significant result ($p < 0.05$) for the probed association with MI based on the primary analysis, as defined by the meta-analysis investigators. The full PubMed search (last update, March 2006) is available upon request. When several meta-analyses had been performed on the same association, we focused on the latest one with more updated information included in the quantitative synthesis for the MI outcome. When no meta-analysis had been performed giving separate calculations for MI in particular, we accepted the latest meta-analysis performed considering all CAD outcomes, and we considered the genetic effect for CAD as a proxy for the genetic effect for MI, unless stated otherwise in the meta-analysis. We considered only those meta-analyses with at least three studies and at least 3,000 cases and controls.

Data extraction. For each eligible meta-analysis, we recorded the author, year of publication, gene, polymorphism, genetic contrast considered in the main analysis, summary odds ratio and 95 percent confidence intervals, ''racial'' descent groups considered, whether there was any mention of differences in the genetic effect across ''racial'' descent groups, the average frequency of the genetic marker of interest in the analyzed Caucasian populations, whether there was significant between-study heterogeneity (based on the Q statistic, as above), whether any studies had been included that had 500 or more cases and 500 or more controls in the genetic contrast analysis adopted by the meta-analysis, and whether these ''large'' studies had found statistically significant results on their own ($p < 0.05$) in this analysis. The cutoff for ''large'' studies was selected a priori, and it is unavoidably a subjective choice, but analyses in other fields (13) have used the cutoff sample size of 1,000 to define a ''large'' study, and we wanted to ensure that an adequate number of both cases and controls were available for analysis. A ''large'' study was defined as having at least 500 cases and 500 controls simply for operational reasons. Current evidence (19) suggests that delineating the genetic epidemiology of complex traits may need sample sizes on the scale of several thousands. We also noted whether mention was made of checking whether small studies differed in their results versus larger studies and what method had been used to test for small study effects. Data were not consistently available from all studies to standardize evaluations of small study effects with the same methods across meta-analyses.

Analyses. For each eligible meta-analysis, we estimated the attributable fraction (AF) for MI (or CAD) in the Caucasian populations based on the reported summary odds ratio (OR) and the average frequency f of the genotype group of interest in the Caucasian populations, as follows:

$$\mathrm{AF} = f(\mathrm{OR}-1)/[1+f(\mathrm{OR}-1)].$$

When OR was per allele, AF included a component for homozygotes and one for heterozygotes.

If these genetic factors are not linked and not interacting with each other, then the sum of these attributable fraction estimates provides the total proportion of the risk of MI explained by these identified genetic markers. This estimate would be inaccurate if there are strongly linked markers and strong interactions (positive or negative epistasis). We also estimated the probability that it would have a formally statistically significant result at the $\alpha = 0.05$ level (power) of each ''large'' study given its sample size for cases and controls and given the observed frequency of the genetic marker of interest in the control group, if the summary odds ratio genetic effect in the meta-analysis were true (20). The sum of these probabilities across studies (the expected number of studies with statistically significant results) was then compared with the observed number of statistically significant findings (exact binomial probability test). Analyses were conducted by using SPSS 13.0 (SPSS, Inc., Chicago, Illinois), Stata 8.0 (Stata Corporation, College Station, Texas), StatXact (Cytel Inc., Boston, Massachusetts), and MetaAnalyst (Joseph Lau, Boston, Massachusetts) software. All p values are two tailed.

RESULTS

AT1R+1166A/C meta-analysis

Fifty-four publications were considered. Of those, 31 were excluded after full-text scrutiny, thus leaving 23 studies (appendix table 1) for further analysis. Reasons for exclusion were overlap with an eligible study ($n = 3$), no data after attempting to communicate with the authors ($n = 11$), outcome other than MI ($n = 11$), other polymorphism involved ($n = 4$), and reviews ($n = 2$). The eligible studies pertained to 27 distinct study populations (10,180 cases and 17,129 controls). The median sample size was 429 (interquartile range, 339–744). Twenty-one, two, and four studies included populations of Caucasian, African, and Asian descent, respectively. Nine studies mentioned blinding of genotyping concerning the clinical outcome or vice versa, while further genotype validation was mentioned in only three study populations. Hardy-Weinberg equilibrium in the control groups was reported to be violated in one study, and the authors had made no further comment. Six studies made no mention of Hardy-Weinberg equilibrium testing. Our calculations revealed another violation. The frequency of the $*C$ allele across all control groups was 24 percent (95 percent confidence interval (CI): 21, 26), with major differences across ''racial'' descent groups (Caucasians, 28 percent (95 percent CI: 27, 29); Africans, 8 percent (95 percent CI: 7, 9); and Asians, 11 percent (95 percent CI: 5, 19)).

Statistically significant between-study heterogeneity was found for the contrast of alleles, as well as for the contrasts assuming dominant or recessive inheritance ($I^2 = 62$ percent, $I^2 = 53$ percent, and $I^2 = 46$ percent, respectively). The $*C$ allele significantly increased the risk of MI both overall (random-effects OR = 1.13, 95 percent CI: 1.04, 1.23; $p = 0.005$) and in studies of subjects of Caucasian descent (random-effects OR = 1.14, 95 percent CI: 1.05, 1.24). The fixed-effects estimates showed a very small effect (OR = 1.05) with borderline statistical significance ($p = 0.04$) (table 1, figure 1). The available data suggested differential effects of the $*C$ allele for Africans, but data for

TABLE 1. Summary odds ratios and 95% confidence intervals for various contrasts in the *AT1R*+1166 A/C* meta-analysis by ''racial'' group

	No. of studies (total sample size)	Random effects		Fixed effects		*Q* statistic
		OR*	95% CI*	OR	95% CI	
C vs. *A* allele	26 (52,504)†	1.13	1.04, 1.23	1.05	1.00, 1.09	63.52
Caucasian	20 (48,662)	1.14	1.05, 1.24	1.05	1.00, 1.09	49.22
African	2 (1,866)	0.58	0.32, 1.05	0.59	0.32, 1.07	0.45
Asian	4 (1,976)	1.25	0.69, 2.29	1.23	0.90, 1.68	9.38
CC vs. *AC* + *AA*	26 (26,247)†	1.30	1.09, 1.55	1.14	1.04, 1.25	48.27
Caucasian	20 (24,331)	1.32	1.10, 1.59	1.15	1.04, 1.26	45.53
African	2 (933)	1.41	0.06, 35.05	1.00	0.14, 7.10	1.63
Asian	4 (983)	0.69	0.21, 2.19	0.46	0.11, 1.82	0.36
AC + *CC* vs. *AA*	27 (27,309)	1.11	1.01, 1.22	1.03	0.98, 1.09	53.37
Caucasian	21 (25,388)	1.11	1.01, 1.21	1.03	0.98, 1.09	37.41
African	2 (933)	0.58	0.32, 1.07	0.59	0.32, 1.09	0.27
Asian	4 (988)	1.40	0.70, 2.83	1.61	1.00, 2.59	10.51

* *AT1R*, angiotensin type 1 receptor gene; OR, odds ratio; CI, confidence interval.

† For one study, data were available for the *AC* + *CC* vs. *AA* comparison only (no allele information and no separate data on *AC* and *CC*).

this group were very sparse, and there was no significant between-''racial'' group heterogeneity.

When we restricted our analyses to studies using World Health Organization criteria, the genetic effect (random-effects OR = 1.15, 95 percent CI: 1.05, 1.26) and heterogeneity (I^2 = 40 percent) remained largely unaffected. However, when our analyses were restricted to studies in which any type of blinding was mentioned, both the genetic effect (random-effects OR = 1.01, 95 percent CI: 0.95, 1.07) and heterogeneity (I^2 = 8 percent) were annulled.

In cumulative meta-analysis, the magnitude of the random-effects odds ratio had not changed much over time, but heterogeneity evolved (figure 2). The summary odds ratio changed little between 1994 and 2005, fluctuating between 1.09 and 1.18. Heterogeneity was minimal (I^2 = 3–25 percent) until the end of 1998, when the cumulative data also did not show any formally significant association. Heterogeneity became prominent after 1999, the same time that the summary estimate became formally significant. At that point, a study was published clearly implicating the polymorphism in MI pathogenesis in a Spanish population (21).

The Begg-Mazumdar test showed no correlation (p = 0.98). However, the largest study (8) found absolutely no benefit (OR = 0.96, 95 percent CI: 0.91, 1.02), and this finding was different beyond chance compared with the results of the smaller studies (OR = 1.15, 95 percent CI: 1.05, 1.25). After we excluded the two studies in which Hardy-Weinberg equilibrium was violated, the main results remained unaffected (OR = 1.14, 95 percent CI: 1.04, 1.25); also, correction for Hardy-Weinberg equilibrium deviation did not affect the results (OR = 1.13, 95 percent CI: 1.04, 1.23).

Overview of meta-analyses

A total of 15 polymorphisms (including *AT1R+1166 A/C*) have had an eligible meta-analysis, suggesting that they are significant determinants of the risk of MI or CAD (when separate data for MI were not available) (table 2) (5, 6, 22–28). The 15 polymorphisms pertained to 12 different genes, and they are not known to have any considerable linkage disequilibrium among them. Each meta-analysis included five to 53 studies and 4,067–35,892 subjects (cases and controls combined). Three meta-analyses included only Caucasian populations, whereas the others had some ''racial'' diversity across the included studies; however, this diversity typically was largely limited to Caucasian and Asian populations, with sparse or no data on other ''racial'' descents. One meta-analysis (23) found a much larger genetic effect in studies conducted in Asia versus other studies. Five meta-analyses (5, 6; the current meta-analysis) performed separate analyses according to blinding of genotyping, and two of them found significantly different results depending on whether blinding was stated in the studies (6; the current meta-analysis).

The summary odds ratio estimates were modest. With one exception (OR = 1.73), they all ranged from 0.8 to 1.34. Given these odds ratio estimates and the observed frequencies of the genetic marker of interest, the attributable fraction for MI ranged from less than 1 percent to 7 percent for each polymorphism. However, if these effects are independent, the total attributable fraction for MI would be 42 percent even without considering the two polymorphisms where the minor allele was protective (table 2). Despite the seemingly large sample size, the frequency of the genetic marker of interest was less than 10 percent in the control group in six meta-analyses, and the total number of controls with the genetic marker of interest was less than 600 in seven meta-analyses. With two exceptions, all meta-analyses had examined the possibility that small studies found different results than larger studies, and the meta-analysts had carefully commented on some evidence of this finding in 10 of the 13 meta-analyses. Analyses for claiming

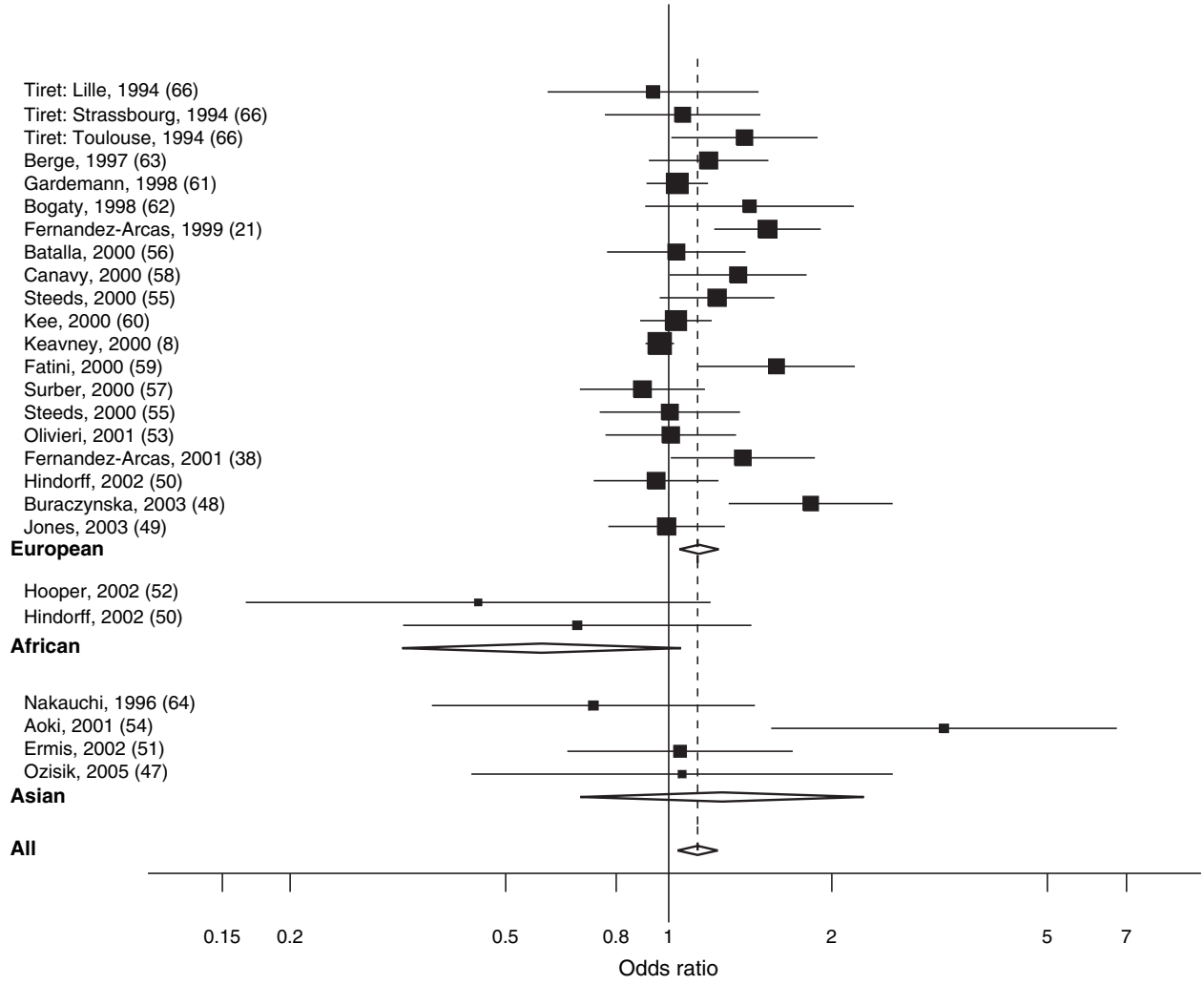

FIGURE 1. Odds ratios for the association between the **C* allele of the *+1166A/C* polymorphism of the angiotensin type 1 receptor gene (*AT1R*) and myocardial infarction. Individual studies are listed by first author, year of publication, and reference number. Diamonds, random-effects summary odds ratios per racial group and overall; horizontal lines, 95% confidence interval. The sizes of the boxes roughly correspond to study weight.

small study effects used different approaches in these meta-analyses with variable credibility and methodological robustness. These approaches included the rather subjective visual inspection of a funnel plot in one meta-analysis (27), simple comparisons of effects in small studies versus larger studies in six (6, 22, 26; the current meta-analysis), and formal correlation and/or regression tests in another six meta-analyses (5, 23, 25).

There were 32 ''large'' studies on these 15 associations (table 2). Only two found formally statistically significant results. Assuming the summary odds ratio estimates, given the study sample sizes and the frequencies of the genetic markers of interest in the control groups, one would expect nine of these ''large'' studies to have formally statistically significant results—far less compared with the two statistically significant studies observed ($p = 0.005$).

DISCUSSION

An evaluation of the large-scale evidence on associations of candidate genes with MI and CAD showed that considerable bias is probably prevalent in this literature. Fifteen associations have been seemingly replicated with large-scale evidence to date. They typically pertained to associations supported by epidemiologic evidence on cardiovascular risk factors and biologic evidence about potentially important molecules rather than being a random selection from the genome. If true, these associations might cumulatively already explain more than a third of the population attributable risk for these outcomes, unless negative epistasis is operating on these gene effects. Moreover, several other associations have been postulated in the recent literature in prestigious journals, and they await further replication (11, 12, 29). Conversely, one would expect that most genetic variants of

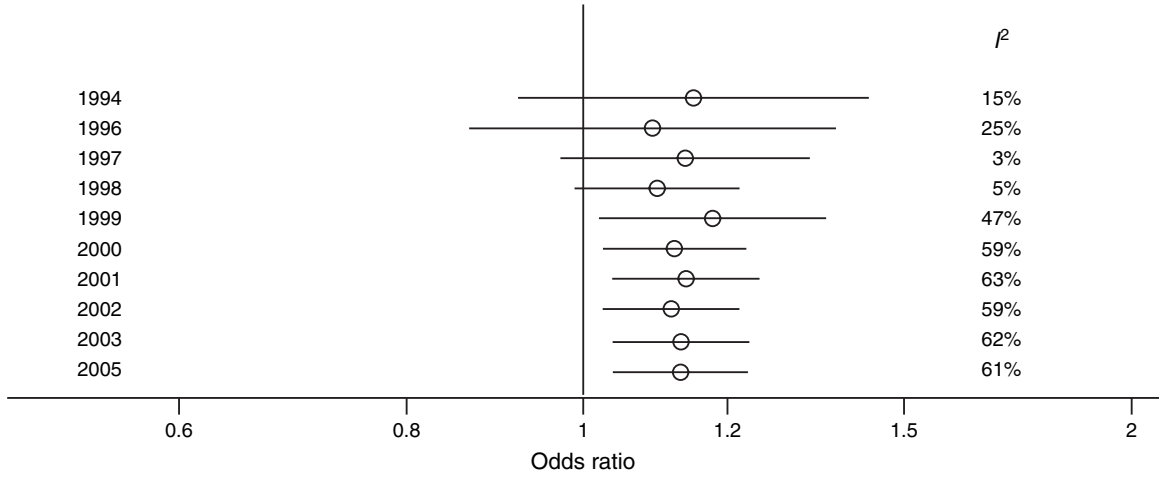

FIGURE 2. Cumulative meta-analysis of the association between the **C* allele and myocardial infarction. The circles show the cumulative odds ratio (random effects) at the end of each year. Also shown are the values of the heterogeneity metric I^2 at the end of each year.

MI are not yet known, since only a negligible portion of the several million polymorphisms of the human genome have been adequately studied in relation to MI to date.

Closer scrutiny of these seemingly replicated associations suggests that many of them may be false positives despite the large amount of accumulated data. First, most of these meta-analyses exhibit large between-study heterogeneity, which could represent genuine variability or bias. However, reasons for genuine variability are not obvious, and most of the available data do not suggest ''racial''-specific effects (30) or other discernible sources of true variability, although exceptions may definitely occur (11, 23). The implicated genes are generally unlinked to each other, although negative and/or positive epistatic interactions cannot be ruled out. Another explanation for genuine heterogeneity is variable gene-environment interactions across different populations, although not documented in any of these associations.

In many of these meta-analyses, the primary meta-analysts carefully noted that the larger studies tend to give more conservative or even entirely ''null'' results. We also observed the same pattern in the case-study meta-analysis of *AT1R+1166A/C*. Moreover, we found that, overall, only two of the 32 largest studies in this field reported formally significant associations. One would have expected that, given their sample sizes, about a third of these large studies should have yielded formally significant results if the estimated associations were true. This pattern suggests that the genetic epidemiology of MI is susceptible to bias. To date, most candidate polymorphisms postulated as being associated with MI have been subsequently contradicted given large-scale evidence (6, 8, 31). Our data suggest that, even among seemingly replicated associations and large-scale evidence, the false-positive rate may still be high.

The exact sources of bias are difficult to dissect. Bias could affect single studies or the field at large. Every genetic association study should be thoroughly assessed for susceptibility to bias. Biologic plausibility, publication bias, selection bias, spectrum of disease bias, population stratification, biased selection of controls, lack of blinding in the genotyping process, and genotyping error are some of the issues that should be considered. In the *AT1R+1166 A/C* meta-analysis, an association was seen only in those studies that did not report blinding, although lack of reporting does not preclude that some of these studies might have been blinded. Information on validation of genotyping and genotyping error has been very limited in genetic association studies to date. However, even modest differential misclassification error could create a spurious odds ratio in the range of those observed here for the putative replicated associations.

The greatest threat may come from bias that affects the field at large. Included are selective reporting bias and publication bias (32, 33). Possibly, investigators may perform analyses by using different definitions of outcomes and different outcomes, and occasionally they may report only the most significant results. In our case-study meta-analysis, we tried to retrieve information from all investigators where MI data for this polymorphism seemed available. Similar, if not more extensive efforts have been conducted for several of the other meta-analyses that have been generally performed by very experienced meta-analyst teams. However, unavoidably, some information will not be retrieved, and other information may remain totally unpublished and inaccessible. Evidence from other fields (34) suggests that selective reporting of outcomes and studies may be a threat regarding the validity of proposed associations, in particular associations of modest size.

In the face of limited validity even for large-scale epidemiologic data, one might seek some additional lines of evidence to sort out the true associations. Biologic plausibility is one such possibility (35). For example, *AT1R+1166 A/C* has weak biologic plausibility. It is located on a noncoding region; there is no relation between the *AT1R* polymorphism and *AT1R* density and/or affinity; plasma levels of renin, angiotensinogen, the angiotensin-converting enzyme gene

TABLE 2. Eligible meta-analyses with formally statistically significant associations

First author, year of publication (reference no.)	Gene polymorphism*	*N*† (no. of subjects)	Genetic contrast	OR†	95% CI†	Descent	Attributable fraction (Caucasian descent)	*Q* statistic	Small study effects	Large studies (SS†)
Myocardial infarction outcome										
Agerholm-Larsen, 2000 (22)	*ACE*† *I/D*	19 (18,664)	*DD* vs. *DI* + *II*	1.21	1.11, 1.32	Caucasian	7%	S†	Yes	1 (0)‡
Song, 2004 (23)	*APOE*† ε	23 (25,470)	$\varepsilon 4/\varepsilon 4 + \varepsilon 3/\varepsilon 4$ vs. $\varepsilon 3/\varepsilon 3$	1.18	1.05, 1.33	Various	3%	S	Yes	2 (1)
Wheeler, 2004 (6)	*PON1*† *Q192R*	19 (13,786)	Allele	1.08	1.02, 1.14	Various	4%	S	Yes	4 (0)
Ye, 2006 (5)	*F5*	53 (35,892)	Allele	1.22	1.10, 1.35	Various	1%	S	Yes	5 (0)
	Prothrombin G20210A	30 (20,567)	Allele	1.25	1.05, 1.50	Various	<1%	S	Yes	4 (0)
	PAI-1† *5G/4G*	31 (21,936)	Allele	1.04	1.00, 1.09	Various	4%	S	Yes	4 (0)
The current meta-analysis, 2007	*AT1R*†+*1166A/C*	26 (26,252)	Allele	1.13	1.04, 1.23	Various	7%	S	Yes	3 (0)
Coronary artery disease outcome										
Boekholdt, 2005 (24)	*CETP*† *TaqIB*	7 (11,672)	*B2B2* vs. *B1B1*	0.77	0.66, 0.89	Caucasian	Protective	NS†	Not checked	1 (1)
Casas, 2004 (25)	*eNOS*† *Glu298Asp*	14 (12,142)	*Asp*/*Asp* vs. others	1.31	1.13, 1.51	Various	3%	S	No	2 (0)
	eNOS intron4 α	16 (12,949)	$\alpha\alpha$ vs. others	1.34	1.03, 1.75	Various	1%	NS	Yes	2 (0)
Chiodini, 2003 (26)	*ApoB*† *XbaI*	20 (6,077)	*TT* vs. others	1.19	1.01, 1.39	Various	6%	S	Yes	0 (0)
	ApoB EcoRI	15 (3,870)	*AA* vs. others	1.73	1.19, 2.50	Various	2%	NS	No	0 (0)
	ApoB Sp Ins/Del	22 (11,616)	*DD* vs. others	1.19	1.05, 1.35	Various	2%	NS	No	2 (0)
Klerk, 2002 (27)§	*MTHFR*† *C677T*	40 (23,920)	*TT* vs. *CC*	1.16	1.05, 1.28	Various	2%	S	Yes	1 (0)
Wittrup, 1999 (28)	*LPL*† *Ser447Ter*	5 (4,067)	*Ter* carriers	0.8	0.7, 1.0	Caucasian	Protective	NS	Not checked	1 (0)

* Gene and polymorphism nomenclature follows the conventions used by each meta-analysis.

† *N*, number of studies included in the meta-analysis; OR, odds ratio; CI, confidence interval; SS, number of statistically significant large studies ($p < 0.05$); ACE, angiotensin-converting enzyme; S, significant between-study heterogeneity ($p < 0.10$); APOE, apolipoprotein E; PON1, paraoxonase 1; PAI-1, plasminogen activating inhibitor; AT1R, angiotensin type 1 receptor; CETP, cholesteryl ester transfer protein, plasma; NS, nonsignificant between-study heterogeneity; eNOS, endothelial nitric oxide synthase; ApoB, apolipoprotein B; MTHFR, methylenetetrahydrofolate reductase; LPL, lipoprotein lipase.

‡ We consider here the study by Keavney et al. (8), the largest on this association not included in the myocardial infarction–specific meta-analysis by Agerholm-Larsen et al. (22). Keavney et al. also performed an extended meta-analysis including unpublished data, but it did not provide data on the sample size of each of the studies included.

§ A more updated meta-analysis on *MTHFR C677T* and coronary artery disease has been published by Lewis et al. (66), but it does not provide data on the sample size of each of the studies included; the summary odds ratio estimate is nevertheless very similar (1.14).

(*ACE*), angiotensin II, or plasminogen activator inhibitor type 1 gene (*PAI-1*) levels are unaffected by the *AT1R* genotype (36–39); and genome scans do not implicate this region in MI linkage (40). However, it is very unlikely that even the majority of true genetic associations would be found in genome-wide linkage studies of the sort mainly carried out to date; they are too small to be detected. Other candidate polymorphisms in the list of the 14 examined here—for example, factor V Leiden (5)—have stronger plausibility based on known biologic effects, but the meaning of biologic plausibility can still be interpreted subjectively. Moreover, in the current era of massive polymorphism testing, it is likely that the identified signals will reflect simply loci in linkage disequilibrium rather than the prime culprit per se. Therefore, biologic support may be seemingly soft or absent for many genuinely promising signals.

We should also caution that we limited our evaluation to seemingly replicated associations with large-scale evidence. Besides significant associations in single or few studies not subjected yet to meta-analysis, the list of tested candidate gene variants with preliminary ''negative'' or inconclusive evidence is much longer. It is possible that evidence on some true genetic risk factors may still be nonsignificant because of underpowered studies. However, it seems that too many spurious associations have already been claimed to date, even when we focus on the most prominently replicated ones.

We conclude that, even when meticulous accumulation of large-scale evidence shows a formally statistically significant association of a polymorphism with MI, the credibility of this association should be viewed with reservation (41). Although a true effect is still possible, the observed small effect may be a measure of the bias in the field (41). Studies of the genetic determinants of MI need to follow rigorous criteria in the design, conduct, analysis, and comprehensive presentation of their results. In addition to the retrospective generation of large-scale evidence through meta-analyses, one has to consider ways to improve the credibility of these associations.

One solution is a consortium, where all investigators working on the genetics of MI participate (42) provided they meet certain criteria for the quality of the design and conduct of their studies and genotyping accuracy. Given the strong tradition of collaborative research in MI genetics (5, 6, 8), this next step should be feasible. The consortium approach should ensure that proposed associations can be assessed within a framework in which definitions and analytical plans are commonly agreed upon, genotyping error is minimized through central quality control, and selective reporting is avoided or even entirely obviated through prospective replication of proposed associations, thus attempting to minimize the presence of spurious heterogeneity. If strong epistasis exists, it is possible that even large-scale studies may fail to detect any strong, consistent associations (43). Complex gene-environment interactions would pose an additional challenge (44, 45). Finally, for certain disease entities, a truly large heritable background may not exist, and their epidemiology may be governed by random, nonsystematic, environmental incidents (46). Even very large-scale consortia would make marginal discoveries at best under such circumstances.

ACKNOWLEDGMENTS

Conflict of interest: none declared.

REFERENCES

1. Fischer M, Broeckel U, Holmer S, et al. Distinct heritable patterns of angiographic coronary artery disease in families with myocardial infarction. Circulation 2005;111:855–62.
2. Yang Q, Khoury MJ, Friedman J, et al. How many genes underlie the occurrence of common complex diseases in the population? Int J Epidemiol 2005;34:1129–37.
3. Lohmueller KE, Pearce CL, Pike M, et al. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177–82.
4. Ioannidis JP, Ntzani EE, Trikalinos TA, et al. Replication validity of genetic association studies. Nat Genet 2001;29:306–9.
5. Ye Z, Liu EH, Higgins JP, et al. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. Lancet 2006;367:651–8.
6. Wheeler JG, Keavney BD, Watkins H, et al. Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. Lancet 2004;363:689–95.
7. Ioannidis JP, Trikalinos TA, Ntzani EE, et al. Genetic associations in large versus small studies: an empirical assessment. Lancet 2003;361:567–71.
8. Keavney B, McKenzie C, Parish S, et al. Large-scale test of hypothesized associations between the angiotensin-converting-enzyme insertion/deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls. Lancet 2000;355:434–42.
9. Marchini J, Donnelly P, Cardon LR. Genome-wide strategies for detecting multiple loci that influence complex diseases. Nat Genet 2005;37:413–17.
10. Palmer LJ, Cardon LR. Shaking the tree: mapping complex disease genes with linkage disequilibrium. Lancet 2005;366:1223–34.
11. Helgadottir A, Manolescu A, Helgason A, et al. A variant of the gene encoding leukotriene A4 hydrolase confers ethnicity-specific risk of myocardial infarction. Nat Genet 2006;38:68–74.
12. Cohen JC, Boerwinkle E, Mosley TH Jr, et al. Sequence variations in *PCSK9*, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354:1264–72.
13. Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med 1997;127:820–6.
14. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
15. Ioannidis JP, Lau J. Evolution of treatment effects over time: empirical insight from recursive cumulative meta-analyses. Proc Natl Acad Sci U S A 2001;98:831–6.
16. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088–101.
17. Cappelleri JC, Ioannidis JP, Schmid CH, et al. Large trials vs meta-analysis of smaller trials: how do their results compare? JAMA 1996;276:1332–8.
18. Schaid DJ, Jacobsen SJ. Biased tests of association: comparisons of allele frequencies when departing from Hardy-Weinberg proportions. Am J Epidemiol 1999;149:706–11.

19. Ioannidis JP, Trikalinos TA, Khoury MJ. Implications of small effect sizes of individual genetic variants on the design and interpretation of genetic association studies of complex diseases. Am J Epidemiol 2006;164:609–14.
20. Pan ZL, Trikalinos TA, Kavvoura FK, et al. Local literature bias in genetic epidemiology: an empirical evaluation of the Chinese literature. PLoS Med 2005;2:e334.
21. Fernandez-Arcas N, Dieguez-Lucena JL, Munoz-Moran E, et al. The genotype interactions of methylenetetrahydrofolate reductase and renin-angiotensin system genes are associated with myocardial infarction. Atherosclerosis 1999;145: 293–300.
22. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. *ACE* gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol 2000;20:484–92.
23. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 2004;141:137–47.
24. Boekholdt SM, Sacks FM, Jukema JW, et al. Cholesteryl ester transfer protein *TaqIB* variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. Circulation 2005;111:278–87.
25. Casas JP, Bautista LE, Humphries SE, et al. Endothelial nitric oxide synthase genotype and ischemic heart disease: meta-analysis of 26 studies involving 23028 subjects. Circulation 2004;109:1359–65.
26. Chiodini BD, Barlera S, Franzosi MG, et al. *APO B* gene polymorphisms and coronary artery disease: a meta-analysis. Atherosclerosis 2003;167:355–66.
27. Klerk M, Verhoef P, Clarke R, et al. *MTHFR* 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 2002;288:2023–31.
28. Wittrup HH, Tybjaerg-Hansen A, Nordestgaard BG. Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease. A meta-analysis. Circulation 1999;99:2901–7.
29. Cornelis MC, El-Sohemy A, Kabagambe EK, et al. Coffee, *CYP1A2* genotype, and risk of myocardial infarction. JAMA 2006;295:1135–41.
30. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. Nat Genet 2004;36: 1312–18.
31. Sie MP, Sayed-Tabatabaei FA, Oei HH, et al. Interleukin 6-174 g/c promoter polymorphism and risk of coronary heart disease: results from the Rotterdam study and a meta-analysis. Arterioscler Thromb Vasc Biol 2006;26:212–17.
32. Easterbrook PJ, Berlin JA, Gopalan R, et al. Publication bias in clinical research. Lancet 1991;337:867–72.
33. Chan AW, Hrobjartsson A, Haahr MT, et al. Empirical evidence for selective reporting of outcomes in randomized trials: comparison of protocols to published articles. JAMA 2004; 291:2457–65.
34. Contopoulos-Ioannidis DG, Alexiou GA, Gouvias TC, et al. An empirical evaluation of multifarious outcomes in pharmacogenetics: beta-2 adrenoceptor gene polymorphisms in asthma treatment. Pharmacogenet Genomics 2006;16:705–11.
35. Jais PH. How frequent is altered gene expression among susceptibility genes to human complex disorders? Genet Med 2005;7:83–96.
36. Pende A, Giacche M, Castigliola L, et al. Characterization of the binding of the RNA-binding protein AUF1 to the human AT 1 receptor mRNA. Biochem Biophys Res Commun 1999;266:609–14.
37. Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. Kidney Int 1999;56:2173–80.
38. Fernandez-Arcas N, Dieguez-Lucena JL, Munoz-Moran E, et al. Both alleles of the *M235T* polymorphism of the angiotensinogen gene can be a risk factor for myocardial infarction. Clin Genet 2001;60:52–7.
39. Stangl K, Cascorbi I, Stangl V, et al. *A1166C* polymorphism of the angiotensin II type 1 receptor gene and risk of adverse events after coronary catheter interventions. Am Heart J 2000;140:170–5.
40. Chiodini BD, Lewis CM. Meta-analysis of 4 coronary heart disease genome-wide linkage studies confirms a susceptibility locus on chromosome 3q. Arterioscler Thromb Vasc Biol 2003;23:1863–8.
41. Ioannidis JPA. Why most published research findings are false. PLoS Med 2005;2:e124.
42. Ioannidis JP, Gwinn M, Little J, et al. A roadmap for efficient and reliable human genome epidemiology. Nat Genet 2006; 38:3–5.
43. Williams SM, Haines JL, Moore JH. The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings? Bioessays 2004;26:170–9.
44. Garcia-Closas M, Malats N, Silverman D, et al. NAT2 slow acetylation, *GSTM1* null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 2005;366:649–59.
45. Hunter DJ. Gene-environment interactions in human diseases. Nat Rev Genet 2005;6:287–98.
46. Ioannidis JP. Commentary: grading the credibility of molecular evidence for complex diseases. Int J Epidemiol 2006; 35:572–8.
47. Ozisik K, Misirlioglu M, Ulus TA, et al. Renin-angiotensin system polymorphisms and coronary artery surgery patients. Asian Cardiovasc Thorac Ann 2005;13:153–6.
48. Buraczynska M, Pijanowski Z, Spasiewicz D, et al. Renin-angiotensin system gene polymorphisms: assessment of the risk of coronary heart disease. Kardiol Pol 2003;58: 1–9.
49. Jones A, Dhamrait SS, Payne JR, et al. Genetic variants of angiotensin II receptors and cardiovascular risk in hypertension. Hypertension 2003;42:500–6.
50. Hindorff LA, Heckbert SR, Tracy R, et al. Angiotensin II type 1 receptor polymorphisms in the Cardiovascular Health Study: relation to blood pressure, ethnicity, and cardiovascular events. Am J Hypertens 2002;15:1050–6.
51. Ermis C, Tsai MY, Hanson NQ, et al. Angiotensin I converting enzyme, angiotensin II type 1 receptor and angiotensinogen polymorphisms and early myocardial infarction in Turkish population. Thromb Haemost 2002;88:693–4.
52. Hooper WC, Dowling NF, Wenger NK, et al. Relationship of venous thromboembolism and myocardial infarction with the renin-angiotensin system in African-Americans. Am J Hematol 2002;70:1–8.
53. Olivieri O, Stranieri C, Girelli D, et al. Homozygosity for angiotensinogen 235T variant increases the risk of myocardial infarction in patients with multi-vessel coronary artery disease. J Hypertens 2001;19:879–84.
54. Aoki S, Mukae S, Itoh S, et al. Genetic background in patients with acute myocardial infarction. Jpn Heart J 2001;42: 15–28.
55. Steeds RP, Wardle A, Smith PD, et al. Analysis of the postulated interaction between the angiotensin II sub-type 1 receptor gene *A1166C* polymorphism and the insertion/deletion

polymorphism of the angiotensin converting enzyme gene on risk of myocardial infarction. Atherosclerosis 2001;154:123–8.
56. Batalla A, Alvarez R, Reguero JR, et al. Synergistic effect between apolipoprotein E and angiotensinogen gene polymorphisms in the risk for early myocardial infarction. Clin Chem 2000;46:1910–15.
57. Surber R, Sigusch HH, Lehmann MH, et al. Angiotensin II type I receptor gene polymorphism is associated with the severity but not prevalence of coronary artery disease. Clin Genet 2000;58:237–8.
58. Canavy I, Henry M, Morange PE, et al. Genetic polymorphisms and coronary artery disease in the South of France. Thromb Haemost 2000;83:212–16.
59. Fatini C, Abbate R, Pepe G, et al. Searching for a better assessment of the individual coronary risk profile. Eur Heart J 2000;21:633–8.
60. Kee F, Morrison C, Poirier O, et al. Angiotensin II type-1 receptor and ACE polymorphisms and risk of myocardial infarction in men and women. Eur J Clin Invest 2000;30: 1076–82.
61. Gardemann A, Nguyen QD, Humme J, et al. Angiotensin II type 1 receptor *A1166C* gene polymorphism. Absence of an association with the risk of coronary artery disease and myocardial infarction and of a synergistic effect with angiotensin-converting enzyme gene polymorphism on the risk of these diseases. Eur Heart J 1998;19:1657–65.
62. Bogaty P, Robitaille NM, Solymoss S, et al. Atherogenic, hemostatic, and other potential risk markers in subjects with previous isolated myocardial infarction compared with long-standing uncomplicated stable angina. Am Heart J 1998;136: 884–93.
63. Berge KE, Bakken A, Bohn M, et al. A DNA polymorphism at the angiotensin II type 1 receptor (AT1R) locus and myocardial infarction. Clin Genet 1997;52:71–6.
64. Nakauchi Y, Suehiro T, Yamamoto M, et al. Significance of angiotensin I-converting enzyme and angiotensin II type 1 receptor gene polymorphisms as risk factors for coronary heart disease. Atherosclerosis 1996;125:161–9.
65. Tiret L, Bonnardeaux A, Poirier O, et al. Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. Lancet 1994;344:910–13.
66. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of *MTHFR 677C→T* polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ 2005;331:1053.

APPENDIX TABLE 1. Characteristics of studies included in the *AT1R+*1166 A/C* meta-analysis†**

First author, year of publication (reference no.)	Country/"racial" group	Definition of cases	Definition of controls	No. of cases	No. of controls
Ozisik, 2005 (47)	Turkey/Asian	CABG* patients; no WHO* criteria‡	Normal vessels on angiography	18	105
Buraczynska, 2003 (48)	Poland/Caucasian	Men with MI*; no WHO criteria	Healthy persons, mostly blood donors; no family history of CHD*	139	200
Jones, 2003 (49)	United Kingdom/ Caucasian	Middle-aged men; WHO criteria (plus 36 coronary revascularization patients)	Middle-aged men; no MI, stroke, unstable angina, or ECG* abnormalities	167	2,111
Hindorff, 2002 (50)	United States/ Caucasian-African	WHO criteria or CHD death	Random Medicare sampling; no MI, CHD	222	1,733
Ermis, 2002 (51)	Turkey/Asian	Aged <45 years; no WHO criteria	No CHD, DM,* hypertension	102	114
Hooper, 2002 (52)	United States/African	No WHO criteria§	No CHD, MI, thrombosis, stroke	100	100
Fernandez-Arcas, 2001 (38)	Malaga, Spain/Caucasian	WHO criteria	No CHD; normal CXR,* ECG, enzymes	212	180
Olivieri, 2001 (53)	Italy/Caucasian	Angiographically severe CHD; no WHO criteria	No CHD, mainly patients with valvular disease, normal vessels on angiography	247	245
Aoki, 2001 (54)	Japan/Asian	No WHO criteria	Routine visits to clinical centers; no CHD, hypertension, DM; normal ECG, lipids	150	150
Steeds, 2001 (55)	United Kingdom/ Caucasian	Aged <75 years; WHO criteria	Patients aged <75 years hospitalized for noncardiac causes; no CHD	541	507
Keavney, 2000 (8)	United Kingdom/ Caucasian	ISIS-3* trial participants: men aged 30–55 years and women aged 30–65 years; no WHO criteria	Healthy relatives aged 30–64 years; no MI, angina, other heart disease, stroke, bleeding, peptic ulcer	4,486	5,759

Table continues

APPENDIX TABLE 1. Continued

First author, year of publication (reference no.)	Country/"racial" group	Definition of cases	Definition of controls	No. of cases	No. of controls
Batalla, 2000 (56)	Spain/Caucasian	Aged <55 years; WHO MONICA* criteria	Hospital staff, residents, blood donors, aged <55 years; no CHD, DM; no drugs for hyperlipidemia or hypertension	220	200
Surber, 2000 (57)	Germany/Caucasian	MI patients with >50% stenosis of a major coronary vessel (not left main)	Consecutive patients undergoing coronary angiography, no valvular disease, PTCA,* CABG; <50% left main stem disease	208	379
Stangl, 2000 (39)	Germany/Caucasian	WHO or angiographic criteria	Inpatients, non-CHD reason; no CHD, PAD,* vasculitis, any severe disease	78	979
Canavy, 2000 (58)	France/Caucasian	ICU* patients aged 18–65 years; WHO criteria	Healthy blood donors matched for age, sex, and body mass index	201	244
Fatini, 2000 (59)	Italy/Caucasian	WHO criteria and CHD angiographically documented	Healthy university staff, normal ECG and physical examination	123	209
Kee, 2000 (60)	United Kingdom/ Caucasian	Aged 25–64 years; WHO MONICA criteria	Age-matched controls, electoral rolls, GP* lists	849	781
Fernandez-Arcas, 1999 (21)	Spain/Caucasian	WHO criteria	No CHD	272	472
Gardemann, 1998 (61)	Germany/Caucasian	WHO criteria (also all had angiography for various reasons)	No MI, all with angiography for suspected CHD (80%) or restricted function (20%); 68% with angina	1,057	1,187
Bogaty, 1998 (62)	Canada/Caucasian	WHO criteria	Healthy males aged <35 years	50	289
Berge, 1997 (63)	Norway/Caucasian	First MI at age <55 years (men) or <60 years (women); no WHO criteria	Population-based Norwegian Twin Panel; no MI	235	384
Nakauchi, 1996 (64)	Japan/Asian	No WHO criteria	Healthy persons aged <75 years, normal physical examination, ECG; no CHD	91	258
Tiret, 1994 (65)	France/Caucasian	Men aged 25–64 years; WHO MONICA criteria	Age-matched controls, electoral rolls, GP lists	412	543

* *AT1R*, angiotensin type 1 receptor gene; CABG, coronary artery bypass grafting; WHO, World Health Organization; MI, myocardial infarction; CHD, coronary heart disease; ECG, electrocardiogram; DM, diabetes mellitus; CXR, chest radiograph; ISIS-3, Third International Study of Infarct Survival; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; PTCA, percutaneous transluminal coronary angioplasty; PAD, peripheral artery disease; ICU, intensive care unit; GP, general practitioners.

† For additional characteristics of the included studies, refer to appendix table 2.

‡ No WHO criteria: history of MI; or no exact definitions for the alterations in enzymes, ECG, or clinical symptoms; or criteria not reported.

§ MI diagnosed with ECG enzymes (95%) and with thallium scan or cardiac catheterization (5%).

(Appendix table 2 follows)

APPENDIX TABLE 2. Additional characteristics of the studies included in the *AT1R+*1166 A/C* meta-analysis**

First author, year of publication (reference no.)	Blinding	Hardy-Weinberg equilibrium testing	Mean age in years (SD*)	
			Cases	Controls
Ozisik, 2005 (47)	NR*	NR	65 (7)	64 (7)
Buraczynska, 2003 (48)	NR	Yes, no violation	53 (7)	47 (11)
Jones, 2003 (49)	Genotype	Yes, no violation	57 (4)	56 (3)
Hindorff, 2002 (50): Caucasian	NR	Yes, no violation	73 for the whole population (all >65)	
Hindorff, 2002 (50): African	NR	Yes, violation	72 for the whole population (all >65)	
Ermis, 2002 (51)	NR	NR	42 (12)	40 (13)
Hooper, 2002 (52)	NR	Yes, no violation	NR	
Fernandez-Arcas, 2001 (38)	NR	NR	54 (13)	56 (15)
Olivieri, 2001 (53)	Genotype	Yes, no violation	60 (10)	58 (13)
Aoki, 2001 (54)	NR	Yes, no violation	64 (8)	62 (10)
Steeds, 2001 (55): Sheffield	Genotype	Yes, no violation	62 (9)	61 (9)
Steeds, 2001 (55): Leicester	Genotype	Yes, no violation	62 (9)	55 (12)
Keavney, 2000 (8)	Genotype	NR	51 (11)	46 (13)
Batalla, 2000 (56)	NR	Yes, no violation	43 (5)	42 (6)
Surber, 2000 (57)	NR	Yes, no violation	NR	
Stangl, 2000 (39)	NR	Yes, no violation	61 (55–67)†	61 (55–67)†
Canavy, 2000 (58)	NR	Yes, no violation	48 (9)	48 (9)
Fatini, 2000 (59)	NR	Yes, no violation	59 (5)	51 (6)
Kee, 2000 (60)	Clinical	Yes, no violation	57	58
Fernandez-Arcas, 1999 (21)	NR	NR	59	43
Gardemann, 1998 (61)	NR	NR	62 (10)	61 (10)
Bogaty, 1998 (62)	NR	NR	54 (9)	<35
Berge, 1997 (63)	NR	Yes, no violation	<60	NR
Nakauchi, 1996 (64)	NR	Yes, no violation	65	56
Tiret, 1994 (65): Toulouse	Clinical	Yes, no violation	25–64†	
Tiret, 1994 (65): Strasburg	Clinical	Yes, violation in cases	25–64†	
Tiret, 1994 (65): Lille	Clinical	Yes, violation in controls	25–64†	

* *AT1R*, angiotensin type 1 receptor gene; NR, not reported; SD, standard deviation.
† Values separated by dashes indicate range.